

Figure S1. Generation and Characterization of VDJ Knockin Mice, Related to Figure 1

(A) Schematic figure of the targeting strategy used for the generation of V(D)J KI mice. Targeting vector-encoded arms of homology to the C57Bl6 wild-type (WT) mouse IgH locus are indicated in green; the neo cassette (ACN) is indicated in gray flanked by loxP sites indicated in brown. The promoter and the human heavy chain V(D)J sequences are indicated in yellow and red, respectively.

(B) Frequencies of immature (B220lo, IgM+ or IgM-) and recirculating (B220+, IgM+) B cell populations in the BM of control WT and V(D)J KI mice are shown as representative FACS diagrams (upper panels) or as average frequencies of individual mice (lower panels) with lines in graphs indicating the mean of the group.

(C) Representative FACS diagrams of marginal zone B cell populations (CD21hi, CD23lo) and follicular B cells (CD21lo, CD23hi) in spleen (upper panels). Total number of splenocytes (lower left) and total number of B cells (lower right) in the spleen of WT and V(D)J KI mice. Lines in graphs indicate mean of the group.

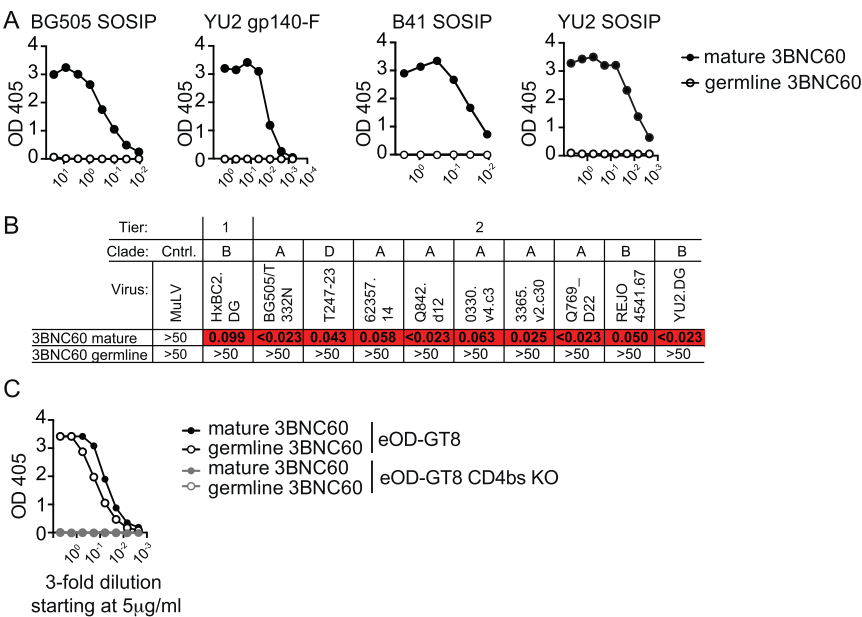


Figure S2. Antigenicity and Neutralization Activity of Predicted Germline and Mature 3BNC60 Monoclonal Antibody, Related to Figure 2

(A) Binding of mature and germline version of 3BNC60 monoclonal antibody against BG505 SOSIP (3-fold dilution starting at 25 μ g/ml), YU2 gp140-F (4-fold dilution starting at 2.5 μ g/ml), B41 SOSIP (3-fold dilution starting at 2.5 μ g/ml), or YU2 SOSIP (3-fold dilution starting at 5 μ g/ml) by ELISA.

(B) Neutralization activity of mature and germline version of 3BNC60 monoclonal antibody against a panel of HIV-1 viruses and control (Cntrl.). Numbers indicate concentration of monoclonal antibody at the median inhibitory concentration (IC_{50}): red, < 0.1 μ g/ml and white, not neutralized at any concentration tested.

(C) ELISA results of mature and germline version of 3BNC60 monoclonal antibody against eOD-GT8 and eOD-GT8 CD4bs knock-out (KO) proteins.

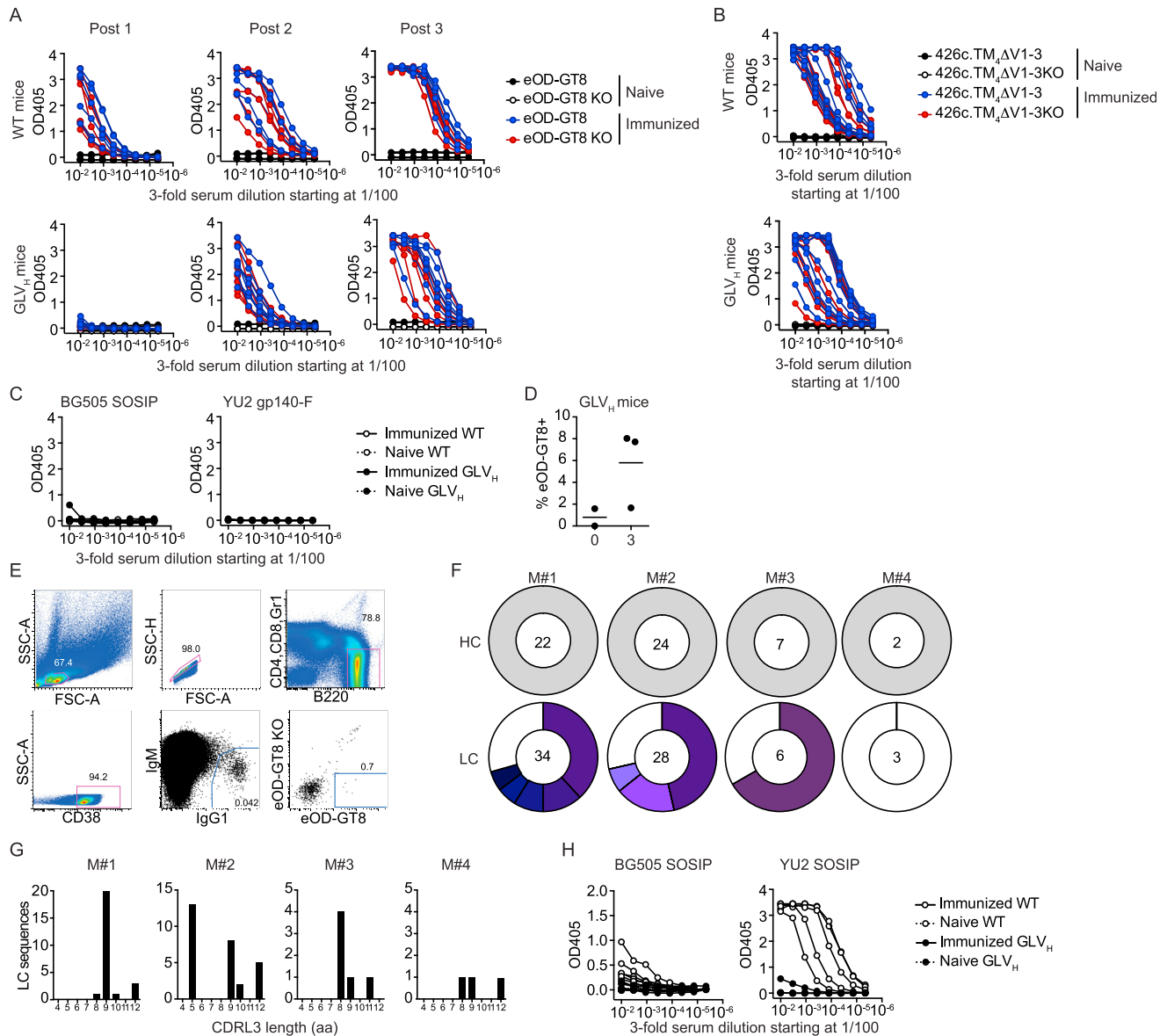


Figure S3. Immunization of GLV_H Mice, Related to Figure 2

(A) Graphs show ELISA titration curves of serum for individual mice against eOD-GT8 and eOD-GT8 CD4bs knock-out (KO) from wild-type C57Bl/6 (WT) and GLV_H mice. Naive serum and serum after one, two or three (Post 1, Post 2 or Post 3) immunizations with eOD-GT8 60-mer are shown.

(B) Graphs show ELISA titration curves of serum for individual mice against 426c.TM₄ΔV1-3 or 426c.TM₄ΔV1-3 CD4bs knock-out (KO) from wild-type C57Bl/6 (WT) and GLV_H mice. Naive serum and serum after one immunization with multimerized 426c.TM₄ΔV1-3 are shown.

(C) Graphs show ELISA titration curves of serum for individual mice against BG505 SOSIP and YU2gp140-F in naive mice or after three immunizations with eOD-GT8 60-mer.

(D) Frequencies of eOD-GT8-specific IgG⁺ memory B cells in individual naive and eOD-GT8 60-mer-immunized GLV_H mice. Lines indicate mean of the individual dots.

(E) Gating strategy for single cell sorting of eOD-GT8 CD4bs-specific memory B cells in eOD-GT8 60-mer-immunized GLV_H mice.

(F) Heavy- (HC) and light-chain (LC) sequences of sorted B cells from individual eOD-GT8 60-mer-immunized GLV_H mice (M# 1, 2, 3 and 4), organized in clones. The number in the center of each pie chart is the number of sequences analyzed; each clone is represented by one slice and its size is proportional to the size of the clone; colors indicate clones (identical V gene and similar CDRL3) and white indicates unique sequences.

(G) CDRL3 aa lengths of cloned LC sequences in (F).

(H) Graphs show ELISA titration curves of serum for individual mice against BG505 SOSIP or YU2 SOSIP in wild-type C57Bl/6 (WT) and GLV_H mice after immunization with BG505 SOSIP or YU2 SOSIP respectively. Naive serum and serum after three- or two immunizations respectively.

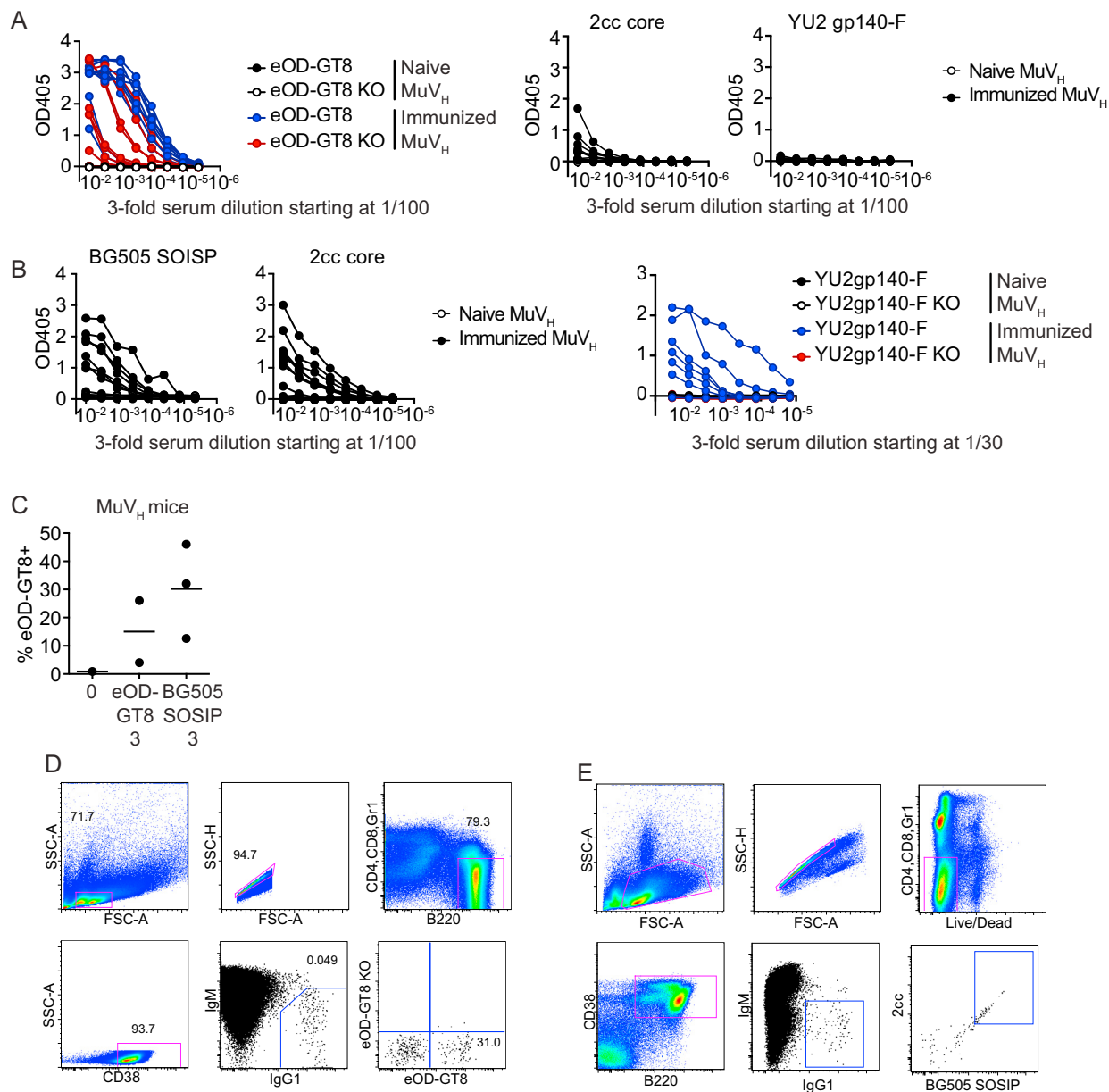


Figure S4. Immunization of MuV_H Mice, Related to Figures 3 and 4

(A) Graphs show ELISA titration curves of serum for individual mice against eOD-GT8 and eOD-GT8 CD4bs knock-out (KO), 2cc core and YU2 gp140-F from naive MuV_H mice or MuV_H mice immunized three times with eOD GT8-60-mer.

(B) As in (A) but after BG505 SOSIP-immunization. ELISA against BG505 SOSIP, 2cc core and YU2 gp140-F and YU2 gp140-F CD4bs knock-out (KO).

(C) Frequencies of eOD-GT8-specific IgG⁺ memory B cells in individual MuV_H mice, naive (0) or after three (3) immunizations with eOD-GT8 60-mer- or BG505 SOSIP. Lines indicate mean of the individual dots.

(D) Gating strategy for single cell sorting of eOD-GT8 CD4bs-specific memory B cells in BG505 SOSIP-immunized MuV_H mice.

(E) Gating strategy for single cell sorting of BG505 SOSIP- and 2cc-specific memory B cells in BG505 SOSIP-immunized MuV_H mice.

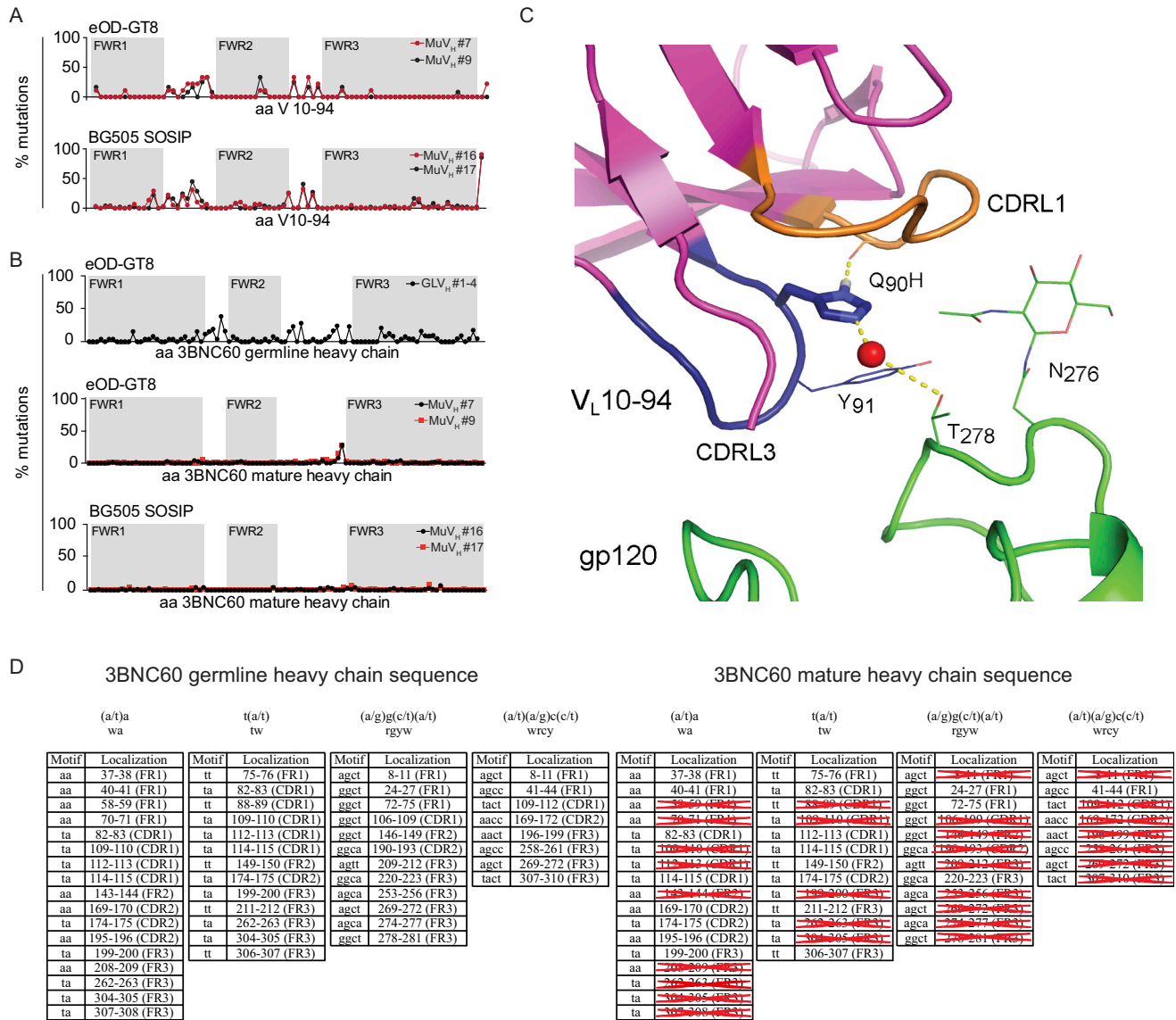


Figure S5. Somatic Hypermutations after Immunization, Related to Figure 5

(A) Light chain somatic mutation analysis of V_L10-94 sequences in eOD-GT8 60-mer- (top) and BG505 SOSIP- (bottom) immunized MuV_H mice. Amino acid (aa) positions of the framework 1-3 (FWR1-3, gray background) and complementarity determining regions (white background) are indicated.

(B) Somatic mutation analysis of the germline (top) and mature (middle and bottom) sequences after immunization with eOD-GT8 60-mer (top and middle) or BG505 SOSIP (bottom).

(C) Structural model of the V_L10-94*01 light chain interacting with Env. V_L10-94*01 light chain interactions in the structural model of an antibody elicited from the MuV_H mouse. A structural model of the complex between 3BNC60 mature heavy chain with an elicited V_L10-94*01 light chain and gp120 is shown (gp120 in green, light chain in magenta, CDRL1 in orange, CDRL3 in dark blue and the heavy chain is hidden for clarity). In the model, the mutation Q₉₀H (shown in sticks) makes hydrogen bonds between the backbone of CDRL1 and an interfacial water molecule (shown as a red sphere). Y₉₁ (shown in lines) packs against the Q₉₀H mutation possibly rigidifying the light chain in a conformation capable of binding gp120. The NAG residue at N276 (shown in lines) is close enough (3.4 Å) in this model to make contact to Q₉₀H.

(D) Mutation hotspots in the germline 3BNC60 heavy chain (HC) compared to the mature 3BNC60 HC sequence. Mutation hotspots in the germline 3BNC60 HC sequence (left). Hotspots that are missing in the mature 3BNC60 HC sequence compared to the germline are crossed out in red (right).